PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 836-115PCT	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/CA2004/000522	International filing date *(day/month/year)* 08.04.2004	Priority date *(day/month/year)* 15.04.2003
International Patent Classification (IPC) or national classification and IPC C07K14/47, G01N33/50, A61K35/00, C12Q1/68		
Applicant XENON GENETICS INC. et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 10 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *sent to the applicant and to the International Bureau)* a total of sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☒	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05.11.2004	30.06.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Telephone No. +49 89 2399- 7846 Seranski, P.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/CA2004/000522

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-60	as originally filed
Claims, Numbers	
1-85	as originally filed
Drawings, Sheets	
1-17	as originally filed
Drawings, Figures	
1-17	as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 33-37, 44-48, 50-54, 84-85 (completely) 38-43, 49 (partially), 14-83, 85

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 33-37, 44-48, 50-54, 84-85 (completely) 38-43, 49 (partially), 14-83, 85

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation to restrict or pay additional fees, the applicant has:
   - ☐ restricted the claims.
   - ☒ paid additional fees.
   - ☐ paid additional fees under protest.
   - ☐ neither restricted nor paid additional fees.
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is
   - ☐ complied with.
   - ☒ not complied with for the following reasons:

     see separate sheet
4. Consequently, this report has been established in respect of the following parts of the international application:
   - ☐ all parts.
   - ☒ the parts relating to claims Nos. 1-14, 62-66, 84 .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-13, 14, 62-66
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-13, 14, 62-66
Industrial applicability (IA)	Yes:	Claims	1-13, 14, 62-66
	No:	Claims	

2. Citations and explanations (Rule 70.7):

   see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

   and / or

2. Non-written disclosures (Rule 70.9)

   see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Re Item III.

Claims 84-85: Rule 39.1(v) PCT - Presentation of information

Re Item IV.

The separate inventions/groups of inventions are:

Invention I: Claims 1-13, 84
Method for identifying a modulating agent for HFE2A gene expression comprising testing a test compound with a genetic construct comprising a reporter gene tinked to the HFE2A promotor; Method for producing test data using said method.

Invention II: Claims 14, 62-66
Method for identifying a modulating agent for HFE2A gene expression comprising testing a test compound with a cell expressing an HFE2A gene.

Invention III: Claims 15-32, 85
Method for identifying a modulating agent for HFE2A activity comprising contacting a test compound with HFE2A polypeptide; Method for producing test data using said method.

Invention IV: Claims 33-54
Method for treating a disorder comprising administering to an animal afflicted therewith a therapeutically effective amount of HFE2A modulator.

Invention V: Claims 55-61
Method to diagnose individuals affected by or at risk of developing a disease of iron metabolism comprising determining a mutation or polymorphism in the nucleic acid sequence of the HFE2A gene.

Invention VI: Claims 67-83
Isolated polynucleotide comprising a polynucleotide having a nucleotide sequence with at least 60% identity to a sequence selected from the group consisting of Seq-ID-N° 1-9, 19-22; isolated polypeptide encoded by said sequences having at least 90% identity to an amino acid sequence selected from the group consisting of Seq-ID-N° 11-12, 23-

28; cell line comprising a recombinant form of said polynucleotide or polypeptide, use of said cell line, composition for treating a disease comprising said polypeptide, method for treating a disease comprising administering said composition.

The groups of inventions not so linked as to form a single general inventive concept (Rule 13.1 PCT) for the following reasons:
The present Application is directed to assay systems for the detection of modulating agents for the activity of the HFE2A gene. The application is based on the discovery that mutations in the HFE2A gene lead to juvenile hemochromatosis. The various assay systems are directed to tests on the promotor activity using a reporter gene in an "in vitro" system (Invention I), to tests on living cells expressing HFE2A and to tests for agents modulating the peptide itself (Invention 3). The only linking technical concept is that all test are related to the HFE2A gene.
The prior art already teaches polypeptides that are identical to the HFE2A polypeptide of the present application (See WO02051438 and AX470382).
In the light of the prior art document D1 different problems can be discerned to underlie the current application, namely the provision test assays either on reporter-gene constructs, living cells or the peptide. The solutions as disclosed and claimed can be summarized as laid down in the claims as grouped in inventions I-III. Invention IV is directed to methods of treatment using the screened agents, thus solving the problem of the provision of a treatment for juvenile hemochromatosis. Invention 5 is directed to a diagnostic method and invention 6 solves the problem of the provision of the polynucleotide encoding the HFE2A polypeptide as such.
In view of the fact that the polypeptide sequence of the HFE2A gene has already been disclosed in the prior art, due to the different problems underlying the current application, and due to the fact that no other technical feature(s) can be distinguished which, in the light
of the prior art, could be regarded as special technical feature(s) common to these solutions, the ISA is of the opinion that there is no single inventive concept underlying the plurality of claimed inventions of the present application in the sense of Rule 13.1 PCT. Consequently there is a lack of unity and different inventions, not belonging to a common inventive concept are formulated as the different subjects on the communication pursuant to Art. 17(3)(a), PCT. "

Re Item V.

The following documents are referred to in this communication:

D1 : WO 02/051438 A (MAX PLANCK GESELLSCHAFT ; STAHL BERND (DE); MACCHI PAOLO (DE); BONHOEF) 4 July 2002 (2002-07-04)

D2 : TAUPIN J-L ET AL: "Functional quantification of cyclosporine A and FK506 in human whole blood by flow cytometry, using the green fluorescent protein as an interleukin-2 reporter gene" JOURNAL OF IMMUNOLOGICAL METHODS, ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, NL, vol. 256, no. 1-2, 1 October 2001 (2001-10-01), pages 77-87, XP004300784 ISSN: 0022-1759

D3 : AFANASSIEV VICTOR ET AL: "Application of yeast cells transformed with GFP expression constructs containing the RAD54 or RNR2 promoter as a test for the genotoxic potential of chemical substances" MUTATION RESEARCH, vol. 464, no. 2, 24 January 2000 (2000-01-24), pages 297-308, XP002302121 ISSN: 0027-5107

D4: GONZALEZ JESUS E ET AL: "Intracellular detection assays for high-throughput screening" CURRENT OPINION IN BIOTECHNOLOGY, vol. 9, no. 6, December 1998 (1998-12), pages 624-631, XP000874867 ISSN: 0958-1669

D5: HERTZBERG R P ET AL: "High-throughput screening: New technology for the 21st century" CURRENT OPINION IN CHEMICAL BIOLOGY, CURRENT BIOLOGY LTD, LONDON, GB, vol. 4, no. 4, August 2000 (2000-08), pages 445-451, XP002256444 ISSN: 1367-5931

D6: MATTHEAKIS L C ET AL: "Expression of Cre recombinase as a reporter of signal transduction in mammalian cells" CHEMISTRY AND BIOLOGY, CURRENT BIOLOGY, LONDON, GB, vol. 6, no. 11, November 1999 (1999-11), pages 835-844, XP002265769 ISSN: 1074-5521

The present application does not meet the criteria of Article 33(1) PCT, because the subject matter of claim 1 does not involve an inventive step in the sense of Article 33(3)PCT.

Document D1, which is considered to represent the most relevant state of the art to the subject matter of claim 1, discloses the sequence of the HFE2A gene of the present Application The subject-matter of independent claim 1 differs from the disclosure of D1 in that an assay system is claimed that involves the use of a reporter gene construct

comprising the promotor sequence of the HFE2A gene. The problem to be solved by the present invention may therefore be regarded as the provision of such an assay system.

In view of D2 and D3 the solution proposed in claim 1 of the present application cannot be considered as involving an inventive step (Article 33(3) PCT) for the following reasons: Both documents, D2 and D3 are cited against the general aspects of such reporter gene assays. They are commonly known to the skilled person. Constructing vectors with promotor sequences from known genes driving the expression of GFP proteins is a standard technique. Without the provision of any further technical effect, a method involving the use of such a construct does not involve an inventive step. The description does neither provide such a construct nor does it provide any indication of a special technical effect that is linked to the use of such a construct in the claimed method.

Therefore the features disclosed in D2 and D3 would be combined by the skilled person, without exercise of any inventive skills in order to solve the problem posed. The proposed solution in independent claim 1 thus cannot be considered inventive (Article 33(3) PCT).

Dependent claims 2-13 do not contain any features which, in combination with the features of the independent claim, meet the requirements of the PCT in respect of inventive step, the reasons being as follows: The features of said claims are merely one of several straightforward possibilities commonly known from which the skilled person would select, without the exercise of inventive skill, in order to solve the problem posed.

The present application does not meet the criteria of Article 33(1) PCT, because the subject matter of claim 14 and 62 does not involve an inventive step in the sense of Article 33(3)PCT. Document D1 representing the closest prior art has already been discussed supra. The subject-matter of independent claim 14 and 62 differs from the disclosure of D1 in that an cell-based assay system is claimed that is used to test the influence of modulating compounds on the expression of the HFE2A gene. The problem to be solved by the present invention may therefore be regarded as the provision of such an assay system.

In view of D4-D6 the solution proposed in claim 14 and 62 of the present application cannot be considered as involving an inventive step (Article 33(3) PCT) for the following reasons: Documents, D4-D6 are cited against the general aspects of such cell-based assay systems. They are commonly known to the skilled person. The use of such cell based assay to evaluating test compound whether they influence gene expression of defined target gene is a standard technique. Without the provision of any further

technical effect, a method involving the use of such a cell-based assay does not involve an inventive step. The description does neither provide such a specific assay nor does it provide any indication of a special technical effect that is linked to the use of such an assay in the claimed method.

Therefore the features disclosed in D4-D6 would be combined by the skilled person, without exercise of any inventive skills in order to solve the problem posed. The proposed solution in independent claims 14 and 62 thus cannot be considered inventive (Article 33(3) PCT).

Dependent claims 63-66 do not contain any features which, in combination with the features of the independent claim, meet the requirements of the PCT in respect of inventive step, the reasons being as follows: The features of said claims are merely one of several straightforward possibilities commonly known from which the skilled person would select, without the exercise of inventive skill, in order to solve the problem posed.